# $N^6$-Cyclopentyl-2-(3-phenylaminocarbonyltriazene-1-yl)adenosine (TCPA), a Very Selective Agonist with High Affinity for the Human Adenosine $A_1$ Receptor

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Four subtypes of adenosine receptors are currently known, that is, $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ receptors. Interestingly, quite substantial species differences exist especially between human and rat $A_3$ receptors. As a result, ligands such as CCPA, which are very selective for the rat $A_1$ receptor versus the human $A_3$ receptor, are substantially less selective when the human $A_1$ and $A_3$ receptors are compared. New 2-substituted and 2,$N^6$-disubstituted adenosines were synthesized, and their affinities for the human adenosine $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ receptors were determined. Although large substituents on the C2-position are generally thought to yield adenosine $A_{2A}$ receptor selective ligands, the reported series of 2-triazeno-substituted adenosines had a very high affinity for the $A_1$ receptor. For example, 2-(3-phenylaminocarbonyltriazene-1-yl)adenosine had an affinity of $6.1 \pm 1.3$ nM for the human adenosine $A_1$ receptor. Introduction of a diphenethyl substituent at the $N^6$-position of this compound resulted in a high-affinity agonist, $3.1 \pm 0.9$ nM, for the human adenosine $A_1$ receptor with 316- and 45-fold selectivity versus the human $A_{2A}$ and human $A_3$ receptors, respectively. The most selective, high-affinity human adenosine $A_1$ receptor agonist was the disubstituted compound $N^6$-cyclopentyl-2-(3-phenylaminocarbonyltriazene-1-yl)adenosine (TCPA). TCPA had an affinity of $2.8 \pm 0.8$ nM for the human adenosine $A_1$ receptor and was 75-fold and 214-fold selective versus the human $A_{2A}$ and human $A_3$ receptors, respectively. In addition, TCPA was a full agonist and inhibited the forskolin-induced cAMP production of CHO cells stably transfected with the human adenosine $A_1$ receptor with an $IC_{50}$ of $1.5 \pm 0.5$ nM.

## Introduction

Four adenosine receptors have been cloned from several species including human. They are named adenosine $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ receptors. Numerous ligands for adenosine receptors have been synthesized and biologically evaluated. For a recent review on adenosine receptors and their ligands see Fredholm et al.[1] Traditionally, selective adenosine $A_1$ receptor agonists were obtained by monosubstitution of the $N^6$-position,[2] whereas introduction of substituents such as alkylamino,[3] alkynyl,[4,5] alkoxy,[6,7] or $N'$-aralkylidenehydrazino[8,9] at the C2-position yielded selective $A_{2A}$ receptor agonists. To date, no selective agonists are available for the $A_{2B}$ receptor. With the discovery in 1991 of the adenosine $A_3$ receptor, the fourth member of the adenosine receptor family, new challenges to synthesize selective ligands arose.[10,11]

The most selective agonist for the human adenosine $A_3$ receptor, $N^6$-(4-aminobenzyl)-5′-methylcarboxamidoadenosine (ABMECA), is substituted on the $N^6$- and 5′-positions. This ligand is very selective for the human $A_3$ receptor versus the other human receptors (70 times over $A_1$ and 166 times over $A_{2A}$). The selective agonist for the human adenosine $A_1$ receptor, 2-Cl-$N^6$-cyclopentyladenosine (CCPA, 2735 times over human $A_{2A}$ and 51 times over human $A_3$),[12] and the selective agonist for the human adenosine $A_{2A}$ receptor CGS21680 (11 times over human $A_1$ and 4 times over human $A_3$, 2-[[4-(2-carboxyethyl)phenethyl]amino]adenosine-5′−$N$-ethylcarboxamide), on the other hand, display considerable affinity for the human $A_3$ receptor.[12] Hence, more selective ligands for both the human adenosine $A_1$ and the human adenosine $A_{2A}$ receptor are required. The objective of this study is to generate agonists for the human adenosine $A_1$ receptor with higher overall selectivity over the other human adenosine receptors. Although substitution at the C2-position is generally thought to yield selective agonists for adenosine $A_{2A}$ receptors, some 2-substituted agonists show preference for the adenosine $A_1$ receptor. Introduction of small groups such as chlorine at the C2-position in combination with the $N^6$-cyclopentyl moiety (CCPA) resulted in the most selective agonist for the human adenosine $A_1$ receptor known to date as shown in this study and by Klotz et al.[12]

In a previous study we have reported on $N^6$-substituted, 2-$NO_2$ analogues of adenosine.[13] The presence of the $NO_2$ group at the C2-position together with a cyclopentyl substituent at the $N^6$-position resulted in a selective ligand for the rat adenosine $A_1$ receptor. To further improve selectivity we have taken 2-nitrosoadenosine as a starting point in our search for

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**Scheme 1.** Synthesis of Carbamoyl Triazenes **8a–l**, **11**, **12**, and **16a–d**[a]
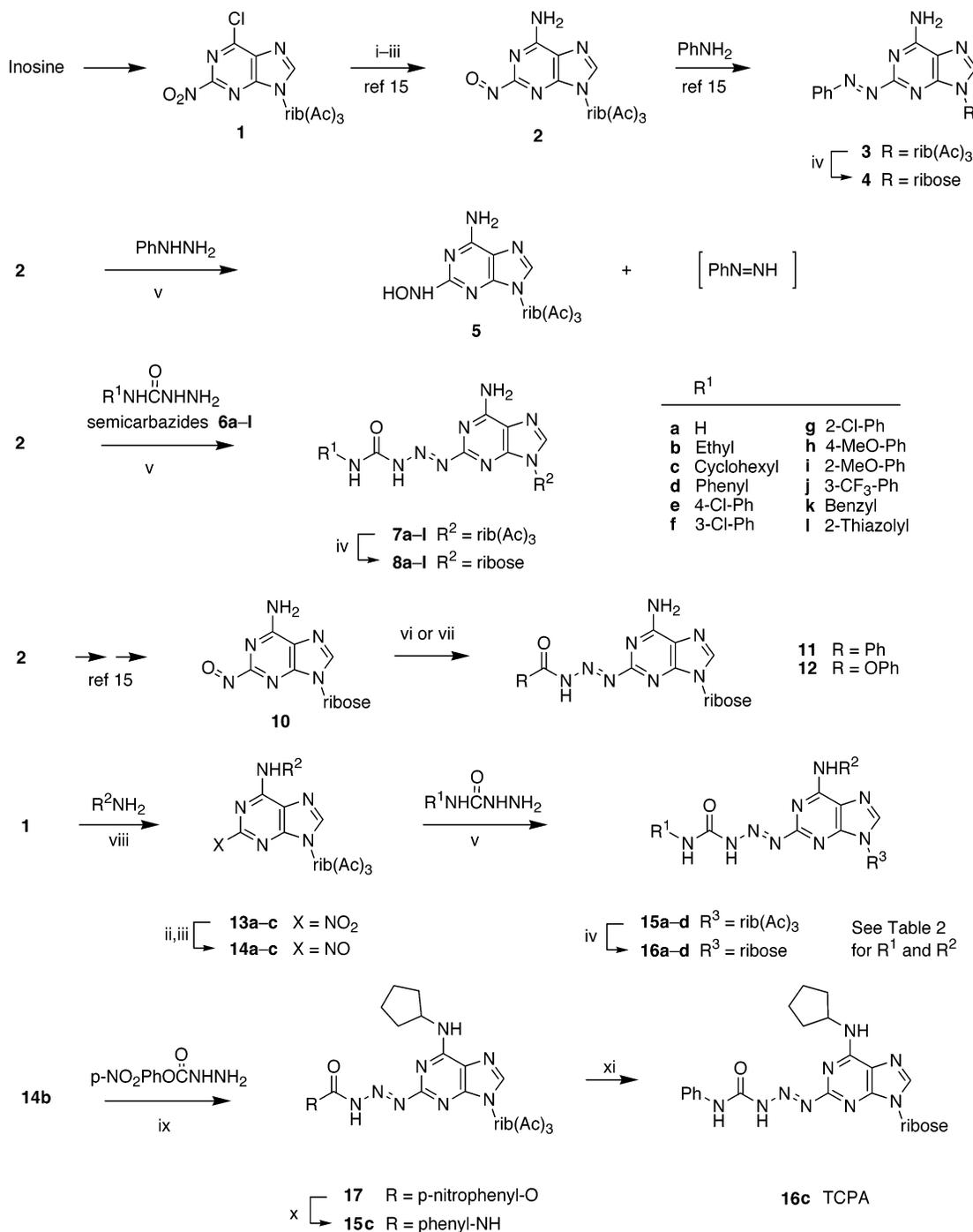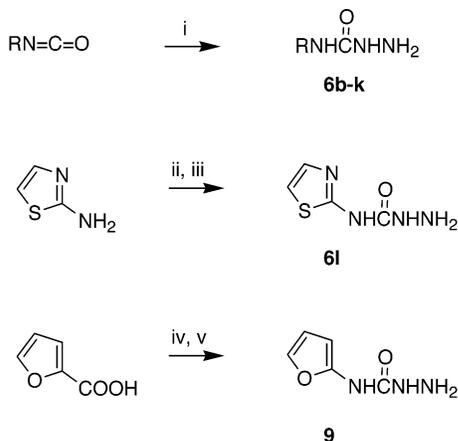


[a] Reagents and conditions: (i) 1.0 equiv of $NaN_3$, DMF, $-18\ °C$; (ii) $H_2$, Pd/C, EtOAc; (iii) $NaIO_4$, EtOAc, $H_2O$; (iv) aq $NH_3$/MeOH; (v) $CH_3CN$/HOAc 10:1; room temperature, 5 h; (vi) **11**: benzoyl hydrazide, $CH_3CN$/HOAc 10:1, room temperature, 18 h, 54%; (vii) **12**: phenyl carbazate, $CH_3CN$/MeOH/HOAc 10:2:1, room temperature, 18 h, 32%; (viii) amine, DMF, DiPEA, room temperature; (ix) DCM/HOAc 10:1, room temperature, 18 h (71%); (x) aniline, room temperature, 8 h (83%); (xi) aq $NH_3$/MeOH (61%).

selective agonists for the human adenosine $A_1$ receptor. These data show that proper substitution at the C2-position yields selective agonists for this receptor. In contrast to small substituents such as chlorine and $NO_2$, the compounds we present here contain large substituents at the C2-position. Combined substitution at C2 and $N^6$ further improves this selectivity.

## Results and Discussion

**Chemistry.** 2-Nitrosoadenosine is a valuable precursor for the attachment of nitrogen-based side chains to

the adenosine 2-position. In particular, the key precursor 6-chloro-2-nitropurine riboside **1** is readily obtained from inosine, via nitration of 6-chloropurine riboside triacetate at the purine 2-position using the tetrabutylammonium nitrate/trifluoroacetic anhydride combination (Scheme 1).[14] Azidation of the 6-position in compound **1** followed by redox conversion of the nitro to the nitroso group produced crystalline **2**.[15] The exceptional reactivity of this nitroso functionality allows condensation with a variety of nitrogen nucleophiles. The first example shown is the acetic acid-catalyzed Mills reac-

**Scheme 2.** Synthesis of Semicarbazides[a]

RN=C=O → (i) → RNHC(=O)NHNH$_2$ **6b–k**

(thiazol-2-amine) → (ii, iii) → **6l**

(furan-2-carboxylic acid, COOH) → (iv, v) → **9**

[a] Reagents and conditions: (i) NH$_2$NH$_2$·H$_2$O, DCM, −60 °C to room temperature; (ii) (PhO)$_2$CO, neat, 100–150°C; (iii) NH$_2$-NH$_2$·H$_2$O, DCM, rT, 18 h; (iv) (PhO)$_2$P(O)N$_3$, DiPEA, DCM, room temperature; (v) PhOH, CH$_3$CN, reflux, 18 h, then NH$_2$NH$_2$.HBr, Et$_3$N, room temperature, 2 h.

tion of **2** with aniline, producing 2-phenyldiazoadenosine **3** in good yield.

Elongation of the two-atom side chain was first examined by condensation of **2** with phenylhydrazine. The strong reducing properties of this type of electron-rich hydrazines, however, completely converted the nitroso group in **2** to hydroxyamine **5**, whereas condensation products could not be isolated. Introduction of electron-withdrawing substituents such as trifluoromethyl and cyano in the phenyl ring of the hydrazine did not give any improvement.

Satisfactory results were obtained with 4-phenylsemicarbazide **6d** as a nucleophile in an acetic acid-catalyzed condensation reaction, producing phenylcarbamoyl-substituted triazene **7d** in 63% yield. Attachment of a carbonyl group directly to the hydrazine obviously decreases the reducing properties of the hydrazine without affecting its nucleophilicity. Only a few examples of reactions between nitrosobenzenes and semicarbazides are known in the literature, and it appears that the favorable reactivity of **2** makes this reaction a success.[16,17] Removal of the acetates from the ribose in **7d** was readily accomplished with a mixture of methanol and aqueous ammonia.

A series of 4-substituted semicarbazides **6a–l** was synthesized from hydrazine hydrate and the corresponding isocyanates, as is shown in Scheme 2. Because thiazole-2-isocyanate is not commercially available, 4-(2-thiazolyl)semicarbazide **6l** was prepared from 2-aminothiazole in two steps. Furylsemicarbazide **9** could be obtained from furan-2-carboxylic acid via Curtius rearrangement. Condensation of the 4-substituted semicarbazides **6a–l** with **2** gave triazenes **7a–l** in 43–63% yield, and deprotection with aqueous ammonia furnished the corresponding triazenes **8a–l** (60–93% yield). 4-Furylsemicarbazide **9** formed the only exception: instead of the anticipated condensation reaction, a 4+2 hetero-Diels−Alder reaction occurred between the furan ring and the nitroso group, leading to extensive decomposition. This type of cycloaddition has been described before in the literature.[15]

Two additional carbonyl-substituted hydrazines, benzoylhydrazide and phenyl carbazate, were examined

(Scheme 1). A condensation reaction of **2** with both hydrazides proceeded in high yield, but removal of the acetate protecting groups with ammonia resulted in fragmentation of the triazene. Because deprotection under milder conditions (e.g., KCN in methanol) did not prevent this type of decomposition, the deprotection step was avoided by using unprotected 2-nitrosoadenosine **10**. This nitroso compound was prepared in a three-step process from **2** by protection of the nitroso functionality: Diels−Alder reaction with cyclopentadiene, deacylation, and retro-Diels−Alder.[15] Condensation of **10** with benzoylhydrazide and phenyl carbazate directly produced the anticipated triazenes **11** and **12**.

2-Triazenyl-functionalized adenosines with an additional substituent at the N[6]-position were synthesized from 6-chloro-2-nitropurine riboside **1** (Scheme 1). Amination of **1** with aniline, cyclopentylamine, and 2,2-diphenylethylamine gave N[6]-substituted adenosines **13a−c**, respectively.[13] Nitroso derivatives **14a−c** were produced in good yield following the nitro to nitroso reduction/oxidation sequence already applied for the synthesis of **2**. Acetic acid-catalyzed condensation between **14a−c** and semicarbazide **6a** or 4-phenylsemicarbazide **6d** followed by removal of the acetates gave N[6]-substituted triazenes **16a−d**. In particular, for the synthesis of TCPA the condensation step to **15c** was low yielding as a result of nitroso to hydroxylamine reduction induced by 4-phenylsemicarbazide. An improved synthetic procedure was developed for TCPA by triazene formation from the nitroso derivative **14b** (R$^2$ = cyclopentyl) with *p*-nitrophenyl carbazate[18] instead of 4-phenylsemicarbazide (Scheme 1). The electron-withdrawing *p*-nitrophenyl substituent further reduces the reduction potential of the hydrazine without noticeably affecting its nucleophilic properties. Reaction of **17** with aniline followed by aminolysis of the acetate protecting groups produced TCPA (**16c**) in an acceptable 24% yield over six steps.

**Stability Studies.** Because some alkyl- and aryltriazenes are known to decompose slowly in solution, we examined the stability of the carbamoyl-substituted triazenes. No decomposition of N[6]-unsubstituted triazene **8d** and *N*[6]-cyclopentyl-substituted triazene **16c** (TCPA) was found in a 9:1 mixture of 0.05 M phosphate buffer in D$_2$O (pD 7.8) and DMSO-$d_6$ at 25 °C over a period of 5 days, as was monitored by ^{1}H NMR. The stability of these compounds might be due to the presence of electron-withdrawing substituents on both sides of the triazene linker. Heating **8d** and **16c** at 80 °C in aqueous DMSO was necessary before formation of aniline and 2,6-diaminopurine riboside **18** or 2-amino-CPA **19** occurred, as is shown in Scheme 3. In addition, the photostability of the triazene functionality in these compounds was examined. After DMSO-$d_6$ solutions of **8d** and **16c** had been exposed to daylight for several days or to irradiation at 350 nm, no decomposition was observed (^{1}H NMR analysis).

Interestingly, NMR analysis of aqueous solutions of compound **4** shows the presence of a *Z/E* = 41/59 mixture in diffuse daylight; the compound was tested as such. *E* to *Z* isomerization of the N=N double bond has never been observed for the triazenes. In, for example, NMR spectroscopy, only the most stable *E*-isomer is observed.

**Scheme 3.** Stability Studies
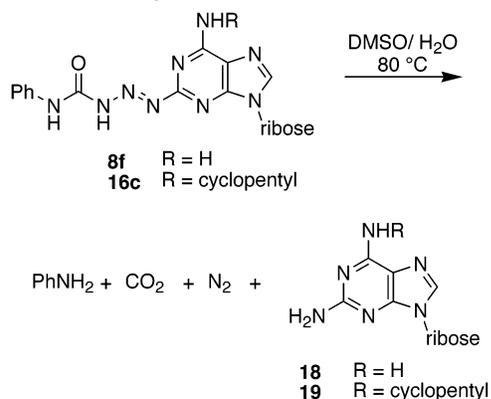


**Biological Evaluation.** All compounds were tested in radioligand binding assays to determine their affinity for the human adenosine receptors. The tritiated antagonist [$^3$H]-1,3-dipropyl-8-cyclopentylxanthine ([$^3$H]-DPCPX) was used for human adenosine $A_1$ and $A_{2B}$ receptors, whereas [$^3$H]-7-amino-2-(2-furyl)-5-[2-(4-hydroxyphenyl)ethyl]amino[1,2,4]triazolo[1,5-*a*][1,3,5]-triazene ([$^3$H]ZM241385) was used as a tritiated antagonist for human adenosine $A_{2A}$ receptors. Due to the lack of commercially available radiolabeled antagonists, we used I$^{125}$-labeled $N^6$-(4-amino-3-iodobenzyl)-5′-methylcarboxamidoadenosine ([$^{125}$I]IABMECA) as a radiolabeled agonist for human adenosine $A_3$ receptors. As we used an antagonist radioligand to determine the affinities of the agonists for the adenosine $A_1$ and $A_{2A}$ receptors, the $K_i$ values represent a combined value for both the high- and low-affinity states of the receptors. The $K_i$ values for the adenosine $A_3$ receptor, on the other hand, represent only the high-affinity state as we used an agonist radioligand to study this receptor.

Our encouraging results to achieve selective adenosine $A_1$ receptor ligands by introducing an $NO_2$ substituent at the C2-position of adenosine prompted us to explore the C2-position in further detail. The $NO_2$ substituent was converted to 2-nitrosoadenosine to obtain a suitable precursor for the attachment of nitrogen-based side chains (Table 1).

Whereas the adenosine $A_3$ receptor was relatively insensitive to the elongation of the side chain, the $A_1$, $A_{2A}$, and, to a lesser degree, $A_{2B}$ receptors preferred compound **8d** with the phenylcarbamoyltriazenyl chain. Extension of the side chain from nitroso (**10**), via diazophenyl (**4**) and benzoyltriazenyl (**11**), to phenyl-carbamoyltriazenyl (**8d**) led to a progressive increase in affinity for the adenosine $A_1$ receptor. Within this series the introduction of an extra nitrogen atom, to obtain **8d**, was most effective and resulted in a 311-fold increase in affinity as compared to **11**. A different pattern was obtained for the other three adenosine receptors. Neither the adenosine $A_{2B}$ receptor nor the adenosine $A_3$ receptor was very sensitive (5-fold affinity change at most) to these C2 substituents. On the other hand, the nitroso-substituted (**10**) and the benzoyltriazenyl-substituted (**11**) analogues had a weak affinity for the adenosine $A_{2A}$ receptor, whereas the diazophenyl-substituted (**4**) compound had a slightly higher affinity. In analogy with the adenosine $A_1$ receptor, the major affinity increase stemmed from the elongation of the spacer with an extra nitrogen atom (**8d**). As a result,

**8d** was selective for the human adenosine $A_1$ receptor with a 4-fold selectivity for $A_1$ versus $A_{2A}$ and an 18-fold selectivity for $A_1$ versus $A_3$.

On the basis of these data, the side chain of **8d** was investigated in greater detail as shown in Table 2. Besides the phenyl substituent (as in **8d**) also several other substituents $R_1$ as well as the unsubstituted (**8a**) compound were tested for their affinity for the human adenosine receptors. Within this series the phenyl (**8d**) and the cyclohexyl (**8c**) compounds had the highest affinity for the adenosine $A_1$ receptor, 6.1 and 5.8 nM, respectively. The unsubstituted compound (**8a**) had a strongly reduced affinity of 140 nM for this receptor. Introduction of an ethyl, benzyl, or thiazole substituent led to a 4−8-fold decrease in affinity. Substitution of the phenyl ring with either a chlorine or methoxy group resulted in a slight 2−4-fold decrease in affinity. Compound **8j** with a trifluoromethyl group at the meta position of the phenyl ring was insoluble in our ligand binding assay buffers. Replacement of one of the nitrogen atoms of **8d** with oxygen resulted in the ester analogue **12**. This alteration was detrimental to the affinity of this compound for the adenosine $A_1$ receptor.

A different pattern was obtained with the adenosine $A_{2A}$ receptor. This receptor had no preference for either a phenyl, ethyl, cyclohexyl, benzyl, or thiazole group at the $R_1$ position, whereas the unsubstituted compound had a 4-fold lower affinity compared to the phenyl-substituted ligand. Substitution of the phenyl group of **8d** was allowed at the ortho position with either a chlorine or a methoxy group or at the para position with a methoxy group and resulted in a 5-fold drop in affinity at most. In contrast, introduction of a chlorine substituent at the meta or para position was not allowed. The ester compound **12** had a very low affinity for the adenosine $A_{2A}$ receptor.
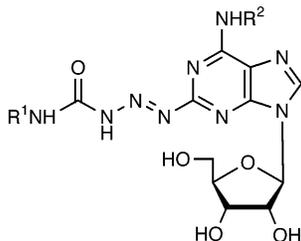
The adenosine $A_3$ receptor was relatively insensitive to the nature of the $R_1$ substituent, with a maximal 2−6-fold change in affinity. Substitution of the phenyl ring was also allowed and resulted in maximal changes in affinity of 3-fold. Even the replacement of nitrogen with oxygen in the spacer as in **12** was tolerated by this receptor.

None of the investigated C2-substituted compounds displayed marked affinity for the adenosine $A_{2B}$ receptor. The highest affinity was obtained with thiazolyl-carbamoyltriazenyladenosine (**8l**) and resulted in a displacement of 79% of radiolabeled [$^3$H]DPCPX at a concentration of 10 $\mu$M. At a concentration of 1 $\mu$M **8l** was able to displace 42% of the radioligand, suggesting that the affinity of **8l** is ∼1 $\mu$M. The affinity of **8l** is comparable to the affinity of NECA (2.2 ± 0.6 $\mu$M; see also Table 2), the reference agonist for the adenosine $A_{2B}$ receptor.[19]

Substitution of adenosine at the $N^6$-position together with a halogen residue at the C2-position has yielded selective high-affinity agonists, such as CCPA, for the adenosine $A_1$ receptor. However, substituents other than halogens at this position are generally detrimental for binding to the adenosine $A_1$ receptor.[20] In addition, C2,$N^6$-disubstituted adenosines with an alkyl chain at the $N^6$-position have a lower affinity compared to the equivalent C2 monosubstituted compounds.[21] Introduction of a cyclopentyl group at the $N^6$-position, however,

**Table 1.** Affinity of 2-Nitrosoadenosine and Analogues for the Human Adenosine $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ Receptors As Determined in Radioligand Binding Studies
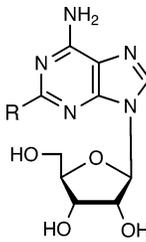


| | | $K_i \pm$ SEM in nM ($n = 3$) or % displacement at 10 $\mu$M ($n = 2$) | | | |
|---|---|---|---|---|---|
| compd | R | $A_1$ | $A_{2A}$ | $A_3$ | $A_{2B}$ |
| **10** | O=N− | 40% | $720 \pm 60$ | $580 \pm 50$ | 14% |
| **4** | phenyl−N=N− | 43% | $180 \pm 10$ | $110 \pm 40$ | 11% |
| **11** | phenyl−CO−NH−N=N− | $1900 \pm 2100$ | $1000 \pm 160$ | $190 \pm 30$ | 8% |
| **8d** | phenyl−NH−CO−NH−N=N− | $6.1 \pm 1.3$ | $25 \pm 3$ | $110 \pm 20$ | 54% |

**Table 2.** Affinities for Human Adenosine Receptors As Determined in Radioligand Binding Studies



| | | | $K_i \pm$ SEM in nM ($n = 3$) or % displacement at 10 $\mu$M ($n = 2$) | | | |
|---|---|---|---|---|---|---|
| compd | $R^1$ | $R^2$ | $A_1$ | $A_{2A}$ | $A_3$ | $A_{2B}$ |
| **8a** | H | H | $140 \pm 34$ | $88 \pm 12$ | $209 \pm 62$ | 9% |
| **8b** | ethyl | H | $45 \pm 17$ | $19 \pm 10$ | $113 \pm 21$ | 15% |
| **8c** | cyclohexyl | H | $5.8 \pm 1.8$ | $25 \pm 3$ | $63 \pm 17$ | 6% |
| **8d** | phenyl | H | $6.1 \pm 1.3$ | $25 \pm 3$ | $110 \pm 20$ | 54% |
| **8e** | 4-Cl-phenyl | H | $15 \pm 11$ | 4.4% | $39 \pm 6$ | 21% |
| **8f** | 3-Cl-phenyl | H | $15 \pm 4$ | 29.9% | $59 \pm 25$ | 64% |
| **8g** | 2-Cl-phenyl | H | $23 \pm 8$ | $45 \pm 19$ | $179 \pm 33$ | 30% |
| **8h** | 4-MeO-phenyl | H | $20 \pm 10$ | $25 \pm 1$ | $73 \pm 11$ | 15% |
| **8i** | 2-MeO-phenyl | H | $20 \pm 3$ | $125 \pm 49$ | $81 \pm 13$ | 18% |
| **8j** | 3-CF$_3$-phenyl | H | insoluble | insoluble | insoluble | insoluble |
| **8k** | benzyl | H | $42 \pm 15$ | $25 \pm 11$ | $147 \pm 18$ | 9% |
| **12** | O-phenyl[a] | H | 43% | 38.9% | $577 \pm 138$ | 6% |
| **8l** | thiazole | H | $24 \pm 1$ | $25 \pm 1$ | $610 \pm 340$ | 79% |
| **16a** | H | phenyl | $204 \pm 136$ | 17.7% | $175 \pm 57$ | 10% |
| **16b** | phenyl | phenyl | $430 \pm 132$ | 24.3% | $90 \pm 12$ | 9% |
| **16c, TCPA** | phenyl | cyclopentyl | $2.8 \pm 0.8$ | $210 \pm 20$ | $600 \pm 230$ | 10% |
| **16d** | phenyl | Ph$_2$CHCH$_2$ | $3.1 \pm 0.9$ | $980 \pm 110$ | $140 \pm 20$ | 10% |
| **CCPA** | | | $6.4 \pm 1.8$ | $639 \pm 55$ | $281 \pm 56^{22}$ | |
| **NECA** | | | $12\ (9.6-15)^b$ | $60 \pm 10$ | $11 \pm 0.8$ | $2200 \pm 600$ |

$^a$ O-phenyl instead of NH-phenyl. $^b$ The $K_i$ value of NECA for the human adenosine $A_1$ receptor was performed in duplicate.

partially restores the affinity of these disubstituted compounds for the adenosine $A_1$ receptor.[20] These data suggest that the binding sites of C2 and $N^6$ substituents might partially overlap. To test this hypothesis, we analyzed carbamoyltriazenyladenosine with a phenyl group at the $N^6$-position (**16a**) and compared the affinity of this compound with that of compound **8d**, in which the phenyl group is present on the triazenyl chain rather than the $N^6$-position. Compound **16a** had a reduced affinity for the adenosine $A_1$ (33-fold) as well as the adenosine $A_{2A}$ receptor (>400-fold), whereas the affinity for the adenosine $A_3$ receptor was hardly affected (2-fold). These data suggest that the C2 and $N^6$ substituents may occupy different binding sites. Moreover, compound **8a**, which lacks a phenyl group at both the C2- and $N^6$-positions, has an affinity comparable to that of **16a** for the adenosine $A_1$ receptor, hence suggesting that disubstitution may be allowed for the adenosine $A_1$ receptor. Indeed, introduction of a phenyl

group at the $N^6$-position of **8d** yielded compound **16b** with an affinity comparable to that of compound **16a** for the adenosine $A_1$ receptor. Substitution of the $N^6$-position with a big substituent, diphenylethyl, further demonstrates that the binding sites of $N^6$ and C2 substituents are different. This compound, **16d**, had a high affinity for the adenosine $A_1$ receptor, whereas the affinity for the $A_{2A}$ receptor dropped considerably compared to that of **8d**. The affinity of **16d** for the $A_3$ receptor was comparable to those of **8d** and **16a**. To exploit the preference of this disubstituted compound for the adenosine $A_1$ receptor, we also introduced the $A_1$ selective cyclopentyl group at the $N^6$-position (**16c**, **TCPA**). TCPA had indeed a high, 2.8 nM, affinity for the adenosine $A_1$ receptor. As anticipated on the basis of radioligand binding data in the rat,[20] the affinity of TCPA for the adenosine $A_{2A}$ receptor was somewhat higher compared to that of the diphenylethyl-substituted compound (**16d**). The 5-fold drop in affinity for the

**Table 3.** Comparison of the Relative Potency and Selectivity of TCPA versus CCPA and NECA for the Human Adenosine $A_1$, $A_{2A}$, and $A_3$ Receptors[a]

| compd | relative potency | | | selectivity | |
|---|---|---|---|---|---|
| | $A_1$ receptor | $A_{2A}$ receptor | $A_3$ receptor | $A_1/A_{2A}$ | $A_1/A_3$ |
| TCPA | 1 (2.8) | 1 (210) | 1 (600) | 75 | 214 |
| CCPA | 0.4 (6.4) | 0.3 (639) | 2.1 (281) | 100 | 44 |
| NECA | 0.2 (12) | 3.5 (60) | 55 (11) | 5 | 0.9 |

[a] The potency of TCPA for the three human adenosine receptors is set at 1. Actual $K_i$ value in nM is presented in parentheses.

adenosine $A_3$ receptor, as compared to **8d**, improved the selectivity of TCPA for the adenosine $A_1$ receptor. The disubstituted compounds had virtually no affinity for the adenosine $A_{2B}$ receptor.

The cyclopentyl- and diphenylethyl-disubstituted compounds, **16c** and **16d**, respectively, are the most selective ligands for the human adenosine $A_1$ receptor known to date due to the improved selectivity with respect to the adenosine $A_3$ receptor. As can be deduced from Table 2, these compounds are 214- and 45-fold selective for the adenosine $A_1$ receptor, respectively. $N^6$-Cyclopentyladenosine, on the other hand, has affinities of $10.2 \pm 1.3$ nM for the human $A_1$ receptor and $281 \pm 56$ nM for the human $A_3$ receptor, resulting in a 28-fold selectivity for the adenosine $A_1$ receptor versus the $A_3$ receptor. 2-Chloro-$N^6$-cyclopentyladenosine (CCPA) has affinities of $6.4 \pm 1.8$, $639 \pm 55$, and $281 \pm 56$ nM[22] for the human adenosine $A_1$, $A_{2A}$, and $A_3$ receptors, respectively (see Table 2). Hence, CCPA is 44-fold selective for the human adenosine $A_1$ receptor versus the human $A_3$ receptor. Although CPA and CCPA are more selective toward the human $A_{2A}$ receptor than TCPA and **16d**, the latter compounds are still 75- and 316-fold selective for $A_1$ versus $A_{2A}$. As we used an agonist radioligand to study ligand binding to the adenosine $A_3$ receptor, and an antagonist radioligand to study binding to the adenosine $A_1$ and $A_{2A}$ receptors, the selectivity toward the adenosine $A_3$ receptor will be underestimated. As a reference, we therefore present the values for the prototypic agonist NECA in Table 2. In our hands, NECA had $K_i$ values for the human adenosine $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ receptors of 12 nM ($9.6-15$ nM; $n = 2$) and $60 \pm 10$, $11 \pm 0.8$, and $2200 \pm 600$ nM, respectively. A comparison of the relative potency and selectivity of TCPA versus CCPA and NECA for the human adenosine $A_1$, $A_{2A}$, and $A_3$ receptors is presented in Table 3.

To verify that TCPA behaves as an agonist, cAMP experiments were performed. Figure 1 shows the inhibition of forskolin-induced cAMP formation in CHO cells stably expressing the adenosine $A_1$ receptor by TCPA and CCPA. Both compounds inhibited the cAMP formation to a similar extent with $IC_{50}$ values of $1.5 \pm 0.5$ and $1.3 \pm 1.1$ nM for TCPA and CCPA, respectively. Hence, in our cAMP assay TCPA is a full agonist like CCPA.

The selectivity of ligands for the adenosine receptors was challenged by two discoveries. First of all, the discovery of the adenosine $A_3$ receptor in 1991[10,11] showed that many ligands with a high affinity for either the human adenosine $A_1$ receptor (e.g., CPA or CCPA) or the human adenosine $A_{2A}$ receptor (e.g., CGS21680) had a relatively high affinity for the human adenosine $A_3$ receptor as well. Second, the affinity of ligands for the adenosine $A_3$ receptor depended strongly upon the
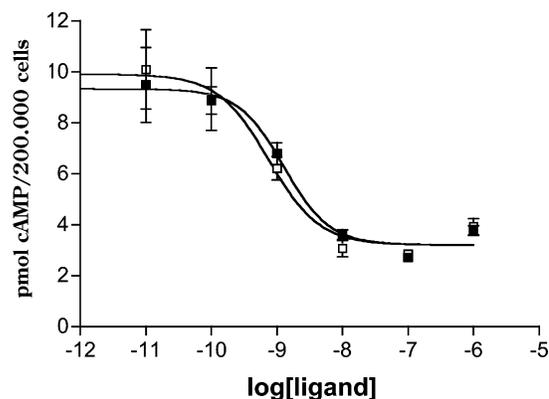


**Figure 1.** Representative curves of the inhibition of forskolin-induced (10 $\mu$M) cAMP production by CCPA ($\square$) and TCPA ($\blacksquare$) in CHO cells stably expressing the adenosine $A_1$ receptor. The amount of cAMP formed is shown. Basal cAMP production was 0.6 pmol/$2 \times 10^5$ cells. The amount of cAMP formed by 10 $\mu$M forskolin amounted to 10.1 pmol/$2 \times 10^5$ cells. After inhibition by CCPA and TCPA, cAMP levels were reduced to 3.9 and 3.8 pmol/$2 \times 10^5$ cells, respectively.

species that was studied. Thus, 2-chloro-$N^6$-(3-iodobenzyl)adenosine-5′-$N$-methyluronamide (Cl-IB-MECA) is a very selective agonist for the rat adenosine $A_3$ receptor versus the rat adenosine $A_1$ (2485-fold) and the rat adenosine $A_{2A}$ receptor (1424-fold).[23] However, considering the human receptors Cl-IB-MECA is only 10-fold (versus $A_1$) and 191-fold (versus $A_{2A}$) selective.[24] In this study we determined the affinity of a series of C2-substituted and C2,$N^6$-disubstituted analogues for the human adenosine receptors. When the affinities for all four human adenosine receptors are compared, TCPA turns out to have the best overall selectivity of the agonists known to date.

## Conclusions

The 2-NO$_2$ analogue of adenosine was converted to 2-nitrosoadenosine to obtain a suitable precursor for the attachment of nitrogen-based side chains. Extension of the nitroso group to diazophenyl, benzoyltriazenyl, and phenylcarbamoyltriazenyl resulted in a compound (**8d**) with low nanomolar affinity for the human adenosine $A_1$ and $A_{2A}$ receptors. Several analogues of **8d** were made, and biological evaluation of these compounds demonstrated that the phenyl substituent was the most appropriate group to achieve a high-affinity adenosine $A_1$ receptor ligand. To further improve the slight preference of **8d** for the adenosine $A_1$ receptor, $N^6$,C2-disubstituted compounds were synthesized. The introduction of diphenylethyl at the $N^6$-position yielded a very selective compound for the human adenosine $A_1$ receptor with 316-fold preference over $A_{2A}$ and 45-fold preference over $A_3$. The disubstituted $N^6$-cyclopentyl-2-phenylcarbamoyltriazene analogue (**16c**, TCPA) was very selective, 75-fold, with respect to the human adenosine $A_{2A}$ receptor. Moreover, TCPA turned out to be the most selective agonist for human adenosine $A_1$ receptors versus human adenosine $A_3$ receptors known to date. Its selectivity for $A_1$ over $A_3$ was 214-fold compared to the 44-fold selectivity of the reference $A_1$ agonist CCPA. This selectivity is most likely underestimated due to the fact that we used an agonist radioligand to study ligand binding to the adenosine $A_3$ receptor, whereas an antagonist radioligand was used to study binding to the

adenosine $A_1$ and $A_{2A}$ receptors. In addition, TCPA turned out to be a full agonist, just like CCPA, and inhibited cAMP production in CHO cells stably transfected with the adenosine $A_1$ receptor with an $IC_{50}$ of $1.5 \pm 0.5$ nM.

## Experimental Section

**Chemicals and Solvents.** All reagents and solvents were used as commercially available, unless indicated otherwise. Adenosine deaminase was from Boehringer Mannheim (Mannheim, Germany), and CGS15943 was a gift from Dr. M. Williams and Dr. J. Watthey (Ciba-Geigy, Summit, NJ). CPA was obtained from RBI Research Chemicals (Natick, MA), and R-PIA was obtained from Sigma (St. Louis, MO).

**Chromatography.** Thin-layer chromatography (TLC) was carried out using silica-coated plastic sheets (Merck silica gel 60 $F_{254}$). Spots were visualized under UV (254 nm). Flash chromatography refers to purification using the indicated eluents and Janssen Chimica silica gel 60 (0.030–0.075 mm).

**Instruments and Analysis.** Proton nuclear magnetic resonance ($^1$H NMR) spectra and carbon nuclear magnetic resonance ($^{13}$C NMR; APT) spectra were determined in CDCl$_3$ at 300 K using a Bruker ARX 400 (400 and 100 MHz, respectively) spectrometer. Mass spectra and accurate mass measurements were performed on a JEOL JMS-SX/SX 102 A tandem mass spectrometer using fast atom bombardment (FAB) or electron impact (EI). A resolving power of 10000 (10% valley definition) for high-resolution electron impact or FAB mass spectrometry was used. Melting points were measured with a Leitz melting point microscope.

**General Procedure for the Synthesis of Semicarbazides 6b–k.** The appropriate isocyanate (10.0 mmol) was added to a mixture of hydrazine hydrate (0.485 mL, 10.0 mmol) and DCM (distilled from P$_2$O$_5$, 25 mL) at −60 °C. The bath was removed and the suspension was stirred for 4 h at room temperature, cooled in ice, and filtered. Additional product was obtained by concentrating the mother liquor. Noncrystalline semicarbazides were obtained by evaporation of the volatiles.

**4-Ethylsemicarbazide 6b:** yield, 0.91 g (88%), obtained as a pure oil after evaporation; $^1$H NMR (DMSO-$d_6$) δ 6.15 (broad, 1H, N–H), 6.0 (broad, 1H, N–H), 3.6 (broad, 2H, NH$_2$), 3.26 (m, 2H, CH$_2$), 1.1 (t, 3H, $J = 7.2$ Hz, CH$_3$).

**4-Cyclohexylsemicarbazide 6c:** yield, 1.35 g (86%), crystallized from ethyl acetate after evaporation of the DCM, mp 100–105°C; $^1$H NMR (DMSO-$d_6$) δ 7.40 (s, 1H, N–H), 5.98 (d, 1H, $J = 7.1$ Hz, N–H), 4.05 (m, 1H, CHNH), 3.60 (broad, 2H, NH$_2$), 1.0–1.8 (m, 10H, cyclohexyl).

**4-Phenylsemicarbazide 6d:** yield, 1.22 g (81%), mp 122–123 °C; $^1$H NMR (DMSO-$d_6$) δ 8.75 (s, 1H, N–H), 7.40 (s, 1H, N–H), 7.2–7.8 (m, 5H, Ar), 4.35 (broad, 2H, NH$_2$).

**4-(4-Chlorophenyl)semicarbazide 6e:** yield 1.65 g (89%), mp 190 °C (dec); $^1$H NMR (DMSO-$d_6$) δ 8.78 (s, 1H, N–H), 7.58 (m, 2H, Ar), 7.48 (s, 1H, N–H), 7.30 (m, 3H, Ar), 4.37 (broad, 2H, NH$_2$).

**4-(3-Chlorophenyl)semicarbazide 6f:** yield, 0.95 g (51%), mp 107–108 °C after recrystallization from DCM/ether; $^1$H NMR (DMSO-$d_6$) δ 8.85 (s, 1H, N–H), 7.81 (s, 1H, Ar), 7.55 (s, 1H, N–H), 7.41 (m, 1H, Ar), 7.25 (t, 1H, $J = 8.1$ Hz, Ar), 6.97 (m, 1H, Ar), 4.39 (broad, 2H, NH$_2$).

**4-(2-Chlorophenyl)semicarbazide 6g:** yield, 1.39 g (75%), mp 110–112 °C after recrystallization from ether/light petroleum; $^1$H NMR (DMSO-$d_6$) δ 9.15 (s, 1H, N–H), 8.30 (m, 1H, Ar), 7.81 (s, 1H, N–H), 7.44 (m, 1H, Ar), 7.29 (m, 1H, Ar), 6.98 (m, 1H, Ar), 4.70 (broad, 2H, NH$_2$).

**4-(4-Methoxyphenyl)semicarbazide 6h:** yield, 1.59 g (88%), mp 145–146 °C; $^1$H NMR (DMSO-$d_6$) δ 8.45 (s, 1H, N–H), 7.41 (d, 2H, $J = 8.7$ Hz, Ar), 7.29 (s, 1H, N–H), 8.82 (d, 2H, $J = 8.7$ Hz, Ar), 4.31 (s, 2H, NH$_2$), 3.71 (s, 3H, CH$_3$O).

**4-(2-Methoxyphenyl)semicarbazide 6i:** yield, 1.60 g (88%), mp 155–157 °C; $^1$H NMR (DMSO-$d_6$) δ 8.90 (s, 1H, N–H), 8.17 (d, 1H, $J = 7.0$ Hz, Ar), 7.62 (s, 1H, N–H), 6.8–7.2 (m, 3H, Ar), 4.52 (broad, 2H, NH$_2$).

**4-(3-Trifluoromethylphenyl)semicarbazide 6j:** yield, 2.00 g (91%), mp 98–99 °C; $^1$H NMR (DMSO-$d_6$) δ 9.02 (s, 1H, N–H), 8.11 (broad, 1H, Ar), 7.76 (m, 1H, Ar), 7.61 (s, 1H, N–H), 7.46 (t, 1H, $J = 7.8$ Hz, Ar), 7.25 (d, 1H, $J = 7.8$ Hz, Ar), 4.41 (broad, 2H, NH$_2$).

**4-Benzylsemicarbazide 6k:** yield, 1.19 g (72%), mp 110–112 °C; $^1$H NMR (DMSO-$d_6$) δ 7.2–7.35 (m, 5H, Ar), 7.03 (s, 1H, N–H), 6.83 (broad, 1H, N–H), 4.26 (d, 1H, $J = 6.0$ Hz, CH$_2$Ph), 4.15 (broad, 2H, NH$_2$).

**4-(2-Thiazolyl)semicarbazide 6l.** A solution of N-(phenoxycarbonyl)-2-aminothiazole (1.1 g, 5 mmol, prepared from 2-aminothiazole[25]) and hydrazine hydrate (0.485 mL, 10 mmol) in DCM (25 mL, distilled from P$_2$O$_5$) was stirred at room temperature for 18 h. Methanol (5 mL) was added, and insoluble material was removed by hot filtration. Evaporation of the filtrate and crystallization of the residue from DCM/ether gave **6e** (0.54 g (68%), mp 162–165 °C; $^1$H NMR (DMSO-$d_6$) δ 10.2 (broad, 1H, N–H), 8.1 (broad, 1H, N–H), 7.34 (d, 1H, $J = 3.5$ Hz, thiazole), 7.06 (d, 1H, $J = 3.5$ Hz, thiazole), 4.25 (broad, 2H, NH$_2$).

**4-(2-Furyl)semicarbazide 9.** Diphenylphosphoryl azide (2.14 g, 10 mmol) was added to a solution of furan-2-carboxylic acid (1.12 g, 10 mmol) and DiPEA (1.918 mL, 11 mmol) in DCM (20 mL, distilled from P$_2$O$_5$). Extractive workup and crystallization from diethyl ether/light petroleum gave the moderately stable furan-2-carbonyl azide (1.23 g, 90%), mp 58–59 °C; $^1$H NMR (DMSO-$d_6$) δ 8.12 (m, 1H, furan), 7.47 (m, 1H, furan), 6.78 (m, 1H, furan). A solution of this azide (0.411 g, 3.0 mmol) and phenol (0.564 g, 6.0 mmol) in CH$_3$CN (10 mL, anhydrous) was refluxed under N$_2$ during 18 h. The reaction mixture was cooled to room temperature and hydrazine·HBr (0.678 g, 6 mmol) and triethylamine (1.6 mL, 12 mmol) were added. After 4 h of stirring at room temperature, the solvent was evaporated and the residue purified by chromatography (ethyl acetate/methanol 9:1) to give **9** (0.180 g, 43%) as a solid, mp >150 °C (dec); $^1$H NMR (DMSO-$d_6$) δ 8.85 (broad, 1H, N–H), 7.50 (broad, 1H, N–H), 7.26 (m, 1H, furan), 6.38 (m, 1H, furan), 5.93 (m, 1H, furan), 4.35 (broad, 2H, NH$_2$).

**General Procedure for the Synthesis of Acetate-Protected Triazenes 7a–l.** To a solution of 2-nitroso-2,3,5-tri-O-acetyladenosine **2** (42 mg, 0.10 mmol) in a mixture of acetonitrile (2 mL) and acetic acid (0.2 mL) was added semicarbazide **6a–l** (0.20 mmol), and the mixture was stirred at room temperature during 4 h. The reaction mixture was diluted with ethyl acetate (10 mL) and stirred with aqueous NaHCO$_3$ (5%, 10 mL) for 30 min. Extractive workup and flash chromatography with 4% methanol in ethyl acetate produced protected triazenes **7a–l** as glass. The products were analyzed by $^1$H NMR and directly used in the deprotection step.

**2′,3′,5′-Tri-O-acetyl-2-(3-aminocarbonyltriazene-1-yl)-adenosine 7a:** yield, 30 mg (63%) after chromatography with 10% methanol in ethyl acetate; $^1$H NMR (DMSO-$d_6$) δ 12.25 (broad, 1H, N–H), 8.37 (s, 1H, H-8), 7.61, (broad, 2H, NH$_2$), 6.5–7.3 (broad, 2H, NH$_2$), 6.21 (d, 1H, $J = 5.1$ Hz, H-1′), 6.03 (m, 1H, H-2′), 5.66 (m, 1H, H-3′), 5.2–5.45 (m, 3H, H-4′, H-5′), 2.16, 2.15, and 2.07 (all 3H, s, acetates).

**2′,3′,5′-Tri-O-acetyl-2-(3-ethylaminocarbonyltriazene-1-yl)adenosine 7b:** yield, 25.7 mg (51%) after chromatography with 7% methanol in ethyl acetate; $^1$H NMR δ 12.3 (broad, 1H, N–H), 8.00 (s, 1H, H-8), 7.04 (s, 1H, N–H), 6.7–7.2 (broad, 2H, NH$_2$), 6.34 (m, 1H, H-2′), 6.09 (d, 1H, $J = 4.4$ Hz, H-1′), 5.72 (m, 1H, H-3′), 4.3–4.5 (m, 3H, H-4′, H5′), 3.47 (m, 2H, CH$_2$CH$_3$), 2.13, 2.09, and 2.02 (all 3H, s, acetates), 1.26 (t, 3H, $J = 7.2$ Hz, CH$_3$).

**2′,3′,5′-Tri-O-acetyl-2-(3-cyclohexylaminocarbonyltriazene-1-yl)adenosine 7c:** yield, 32.9 mg (59%) after chromatography with 4% methanol in ethyl acetate; $^1$H NMR δ 11.9 (s, 1H, N–H), 8.02 (s, 1H, H-8), 7.0 (broad, 2H, NH$_2$), 6.70 (broad, 1H, N–H), 6.23 (m, 1H, H-2′), 6.16 (d, 1H, $J = 5.4$ Hz, H-1′), 5.63 (m, 1H, H-3′), 4.3–4.5 (m, 3H, H-4′, H5′), 3.80 (m, 1H, CHNH), 2.14, 2.07, and 2.04 (all 3H, s, acetates), 1.0–1.9 (m, 10H, cyclohexyl).

**2′,3′,5′-Tri-*O*-acetyl-2-(3-phenylaminocarbonyltriazene-1-yl)adenosine 7d:** yield, 30.0 mg (54%); ^{1}H NMR (DMSO-*d*₆) δ 12.8 (broad, 1H, N−H), 10.1 (broad, 1H, N−H), 8.36 (s, 1H, H-8), 7.65 (m, 4H, Ar−H_ortho, NH₂), 7.35 (t, 2H, *J* = 7.7, Ar−H_meta), 7.09 (t, 1H, *J* = 7.6 Hz, Ar−H_para), 6.21 (d, 1H, *J* = 5.5 Hz, H-1′), 5.99 (m, 1H, H-2′), 5.67 (m, 1H, H-3′), 4.3−4.5 (m, 3H, H-4′, H-5′), 2.10, 2.06, and 1.99 (all 3H, s, acetates).

**2′,3′,5′-Tri-*O*-acetyl-2-[3-(4-chlorophenyl)aminocarbonyltriazene-1-yl]adenosine 7e:** yield, 29.5 mg (50%); ^{1}H NMR (DMSO-*d*₆) δ 12.8 (broad, 1H, N−H), 10.0 (broad, 1H, N−H), 8.36 (s, 1H, H-8), 7.69 (m, 2H, Ar), 7.66 (s, 2H, NH₂), 7.40 (d, 2H, *J* = 8.7 Hz, Ar), 6.21 (d, 1H, *J* = 5.3 Hz, H-1′), 5.98 (m, 1H, H-2′), 5.67 (m, 1H, H-3′), 4.3−4.5 (m, 3H, H-4′, H-5′), 2.10, 2.06, and 1.99 (all 3H, s, acetates).

**2′,3′,5′-Tri-*O*-acetyl-2-[3-(3-chlorophenyl)aminocarbonyltriazene-1-yl]adenosine 7f:** yield, 32.0 mg (54%); ^{1}H NMR δ 12.1 (broad, 1H, N−H), 8.8 (broad, 1H, N−H), 8.03 (s, 1H, H-8), 7.1−7.7 (m, 4H, Ar), 6.0−6.2 (m, 4H, H-1′, H-2′, NH₂), 5.72 (m, 1H, H-3′), 4.3−4.5 (m, 3H, H-4′, H-5′), 2.07, 2.05, and 2.04 (all 3H, s, acetates).

**2′,3′,5′-Tri-*O*-acetyl-2-[3-(2-chlorophenyl)aminocarbonyltriazene-1-yl]adenosine 7g:** yield, 30.0 mg (51%); ^{1}H NMR δ 13.2 (broad, 1H, N−H), 9.03 (broad, 1H, N−H), 8.27 (d, *J* = 7.7 Hz, Ar), 8.08 (s, 1H, H-8), 7.0−7.3 (m, 6H, Ar, NH₂), 6.22 (d, 1H, *J* = 5.1 Hz, H-1′), 5.94 (m, 1H, H-2′), 5.65 (m, 1H, H-3′), 4.3−4.5 (m, 3H, H-4′, H-5′), 2.08, 2.07, and 2.03 (all 3H, s, acetates).

**2′,3′,5′-Tri-*O*-acetyl-2-[3-(4-methoxyphenyl)aminocarbonyltriazene-1-yl]adenosine 7h:** yield, 25.4 mg (43%); ^{1}H NMR δ 11.5 (broad, 1H, N−H), 8.7 (broad, 1H, N−H), 7.99 (s, 1H, H-8), 7.50 (m, 2H, Ar), 7.02 (d, 2H, *J* = 9.0 Hz, Ar), 6.5 (broad, 2H, NH₂), 6.22 (broad, 1H, H-1′), 5.98 (m, 1H, H-2′), 5.71 (m, 1H, H-3′), 4.3−4.5 (m, 3H, H-4′, H-5′), 3.81 (s, 3H, OCH₃), 2.08, 2.04, and 2.02 (all 3H, s, acetates).

**2′,3′,5′-Tri-*O*-acetyl-2-[3-(2-methoxyphenyl)aminocarbonyltriazene-1-yl]adenosine 7i:** yield, 32.1 mg (55%); ^{1}H NMR δ 12.0 (broad, 1H, N−H), 8.70 (broad, 1H, N−H), 8.22 (d, 1H, *J* = 8.3 Hz, Ar), 8.01 (s, 1H, H-8), 7.5 (broad, 2H, NH₂), 6.8−7.1 (m, 3H, Ar), 6.19 (broad, 1H, H-1′), 5.97 (m, 1H, H-2′), 5.70 (m, 1H, H-3′), 4.4−4.5 (m, 3H, H-4′, H-5′), 3.83 (s, 3H, OCH₃), 2.08, 2.06, and 2.03 (all 3H, s, acetates).

**2′,3′,5′-Tri-*O*-acetyl-2-[3-(3-trifluoromethylphenyl)aminocarbonyltriazene-1-yl]adenosine 7j:** yield, 35.0 mg (56%); ^{1}H NMR δ 12.5 (broad, 1H, N−H), 9.90 (broad, 1H, N−H), 8.12 (m, 1H, Ar), 7.96 (m, 3H, H-8, NH₂), 7.2−7.5 (m, 3H, Ar), 6.27 (d, 1H, *J* = 5.1 Hz, H-1′), 5.93 (m, 1H, H-2′), 5.62 (m, 1H, H-3′), 4.4−4.5 (m, 3H, H-4′, H-5′), 2.09, 2.08, and 2.05 (all 3H, s, acetates).

**2′,3′,5′-Tri-*O*-acetyl-2-(3-benzylaminocarbonyltriazene-1-yl)adenosine 7k:** yield, 40.6 mg (71%); ^{1}H NMR δ 11.7 (broad, 1H, N−H), 7.95 (s, 1H, H-8), 7.41 (s, 1H, N−H), 7.2−7.3 (m, 5H, Ar), 6.70 (broad, 2H, NH₂), 6.31 (m, 1H, H-2′), 6.04 (d, 1H, *J* = 4.4 Hz, H-1′), 5.68 (m, 1H, H-3′), 4.66 (m, 2H, *CH*₂-Ph), 4.2−4.5 (m, 3H, H-4′, H5′), 2.04, 2.00, and 1.99 (all 3H, s, acetates).

**2′,3′,5′-Tri-*O*-acetyl-2-[3-(2-thiazolyl)aminocarbonyltriazene-1-yl]adenosine 7l:** yield, 27.0 mg (48%) after chromatography with 4% methanol in ethyl acetate; ^{1}H NMR (DMSO-*d*₆) δ 12.6 (broad, 1H, N−H), 10.8 (broad, 1H, N−H), 8.40 (s, 1H, H-8), 7.71 (s, 2H, NH₂), 7.49 (broad, 1H, thiazole), 7.27 (broad, 1H, thiazole), 6.20 (d, 1H, *J* = 4.4 Hz, H-1′), 5.96 (m, 1H, H-2′), 5.73 (m, 1H, H-3′), 4.40 (m, 1H, H-4′), 4.32−4.5 (m, 2H, H-5′), 2.11, 2.07, and 1.99 (all 3H, s, acetates).

**Deprotection of 7a−l to 8a−l, General Method.** Aqueous ammonia (2 mL of a 25% solution) was added to a solution of triacetate **7a−l** in methanol (2 mL). After 18 h of stirring at room temperature, the solvents were removed in vacuo and the residue was coevaporated with methanol (4 mL). The remaining solid was dried in vacuo (0.1 mbar, 40 °C) for 2 h and triturated with methanol (2 mL), providing the yellow ribosides **8a−l**.

**2-(3-Aminocarbonyl)triazene-1-yl)adenosine 8a:** yield, 18.0 mg (86%), mp 186−188 °C; ^{1}H NMR (DMSO-*d*₆) δ 12.54 (broad, 1H, N−H), 8.35 (s, 1H, H-8), 7.64 (broad, 2H, NH₂),

6.9−7.4 (broad, 2H, NH₂), 5.9 (broad, 1H, OH), 5.85 (d, 1H, *J* = 6.8 Hz, H-1′), 5.45 (d, 1H, *J* = 6.4 Hz, OH), 5.22 (d, 1H, *J* = 4.0 Hz, OH), 4.69 (m, 1H, H-2′), 4.13 (m, 1H, H-3′ or H-4′), 4.04 (m, 1H, H-4′ or H-3′), 3.5−3.7 (m, 2H, H-5′); [found M⁺ + 1, 354.1560; C₁₁H₁₆N₉O₅ requires M, 354.1587]. Anal. Calcd (C₁₁H₁₅N₉O₅·1.0H₂O) C, H, N.

**2-(3-Ethylaminocarbonyltriazene-1-yl)adenosine 8b:** yield, 18.0 mg (93%), mp 172−173 °C; ^{1}H NMR (DMSO-*d*₆) δ 12.40 (s, 1H, N−H), 8.34 (s, 1H, H-8), 7.98 (s, 1H, N−H), 7.65 (s, 2H, NH₂), 6.05 (broad, 1H, OH), 5.86 (d, 1H, *J* = 7.2 Hz, H-1′), 5.45 (d, 1H, *J* = 6.4 Hz, OH), 5.22 (d, 1H, *J* = 3.7 Hz, OH), 4.72 (m, 1H, H-2′), 4.14 (m, 1H, H-3′ or H-4′), 4.06 (m, 1H, H-4′ or H-3′), 3.5−3.7 (m, 2H, H-5′), 3.24 (m, 2H, *CH*₂-CH₃), 1.14 (t, 3H, *J* = 7.2 Hz, CH₃); [found M⁺ + 1, 382.1560; C₁₃H₂₀N₉O₅ requires M, 382.1587]. Anal. Calcd (C₁₃H₁₉N₉O₅·2.1H₂O) C, H, N.

**2-(3-Cyclohexylaminocarbonyltriazene-1-yl)adenosine 8c:** yield, 23.5 mg (92%), mp 164−166 °C; ^{1}H NMR (DMSO-*d*₆) δ 12.35 (s, 1H, N−H), 8.35 (s, 1H, H-8), 7.92 (d, 1H, *J* = 7.1 Hz, N−H), 7.68 (s, 2H, NH₂), 6.20 (broad, 1H, OH), 5.85 (d, 1H, *J* = 7.4 Hz, H-1′), 5.46 (d, 1H, *J* = 6.5 Hz, OH), 5.23 (d, 1H, *J* = 3.4 Hz, OH), 4.79 (m, 1H, H-2′), 4.11 (m, 1H, H-3′ or H-4′), 4.09 (m, 1H, H-4′ or H-3′), 3.5−3.7 (m, 3H, *CH*NH, H-5′), 1.1−1.9 (m, 10H, cyclohexyl); [found M⁺ + 1, 436.2057; C₁₇H₂₆N₉O₅ requires M, 436.2056]. Anal. Calcd (C₁₇H₂₅N₉O₅·1.2H₂O) C, H, N.

**2-(3-Phenylaminocarbonyltriazene-1-yl)adenosine 8d:** yield, 18.0 mg (78%), mp 164−165 °C; ^{1}H NMR (DMSO-*d*₆) δ 12.75 (broad, 1H, N−H), 10.08 (broad, 1H, N−H), 8.37 (s, 1H, H-8), 7.70 (s, 2H, NH₂), 7.60 (m, 2H, Ar−H_ortho), 7.38 (t, 2H, *J* = 7.5 Hz, Ar−H_meta), 7.09 (t, 1H, *J* = 7.5 Hz, Ar−H_para), 6.4 (broad, 1H, OH), 5.88 (d, 1H, *J* = 7.2 Hz, H-1′), 5.49 (d, 1H, *J* = 6.3 Hz, OH), 5.26 (d, 1H, *J* = 3.4 Hz, OH), 4.77 (m, 1H, H-2′), 4.18 (m, 1H, H-3′ or H-4′), 4.07 (m, 1H, H-4′ or H-3′), 3.6−3.8 (m, 2H, H-5′); [found M⁺ + 1, 430.1569; C₁₇H₂₀N₉O₅ requires M, 430.1587]. Anal. Calcd (C₁₇H₁₉N₉O₅·2.2H₂O) C, H, N.

**2-[3-(4-Chlorophenyl)aminocarbonyltriazene-1-yl]adenosine 8e:** yield, 17.0 mg (73%), mp 170−172 °C; ^{1}H NMR (DMSO-*d*₆) δ 12.80 (s, 1H, N−H), 10.13 (s, 1H, N−H), 8.37 (s, 1H, H-8), 7.73 (s, 2H, NH₂), 7.63 (m, 2H, Ar), 7.43 (d, 2H, *J* = 8.7 Hz, Ar), 6.41 (broad, 1H, OH), 5.88 (d, 1H, *J* = 7.1 Hz, H-1′), 5.48 (d, 1H, *J* = 6.3 Hz, OH), 5.26 (broad, 1H, OH), 4.75 (m, 1H, H-2′), 4.19 (m, 1H, H-3′ or H-4′), 4.07 (m, 1H, H-4′ or H-3′), 3.6−3.8 (m, 2H, H-5′); [found M⁺ + 1, 464.1166; C₁₇H₁₉N₉O₅Cl requires M, 464.1198]. Anal. Calcd (C₁₇H₁₈N₉O₅Cl·2.2H₂O) C, H, N.

**2-[3-(3-Chlorophenyl)aminocarbonyltriazene-1-yl]adenosine 8f:** yield, 18.1 mg (78%), mp 159−161 °C; ^{1}H NMR (DMSO-*d*₆) (two rotamers are present; only the major rotamer is shown) δ 12.74 (s, 1H, N−H), 10.03 (s, 1H, N−H), 8.39 (s, 1H, H-8), 7.75 (s, 2H, NH₂), 7.0−7.6 (m, 4H, Ar), 6.46 (d, 1H, *J* = 10.2 Hz, OH), 5.88 (d, 1H, *J* = 7.2 Hz, H-1′), 5.48 (d, 1H, *J* = 6.3 Hz, OH), 5.26 (d, 1H, *J* = 3.3 Hz, OH), 4.80 (m, 1H, H-2′), 4.19 (m, 1H, H-3′ or H-4′), 4.10 (m, 1H, H-4′ or H-3′), 3.6−3.8 (m, 2H, H-5′); [found M⁺ + 1, 464.1186; C₁₇H₁₉N₉O₅Cl requires M, 464.1198]. Anal. Calcd (C₁₇H₁₈N₉O₅Cl·0.5 H₂O) C, H, N.

**2-[3-(2-Chlorophenyl)aminocarbonyltriazene-1-yl]adenosine 8g:** yield, 18.0 mg (76%), mp 160−161°C; ^{1}H NMR (DMSO-*d*₆ δ 12.88 (s, 1H, N−H), 9.43 (s, 1H, N−H), 8.40 (s, 1H, H-8), 7.95 (d, 1H, *J* = 7.3 Hz, Ar), 7.70 (s, 2H, NH₂), 7.2−7.6 (m, 3H, Ar), 5.88 (d, 1H, *J* = 6.8 Hz, H-1′), 5.81 (broad, 1H, OH), 5.46 (broad, 1H, OH), 5.20 (broad, 1H, OH), 4.70 (m, 1H, H-2′), 4.18 (m, 1H, H-3′ or H-4′), 4.12 (m, 1H, H-4′ or H-3′), 3.4−3.7 (m, 2H, H-5′); [found M⁺ + 1, 464.1194; C₁₇H₁₉N₉O₅Cl requires M, 464.1198]. Anal. Calcd (C₁₇H₁₈N₉O₅Cl·1.2H₂O) C, H, N.

**2-[3-(4-Methoxyphenyl)aminocarbonyltriazene-1-yl]adenosine 8h:** yield, 17.4 mg (87%), mp 159−161°C; ^{1}H NMR (DMSO-*d*₆) (two rotamers are present; only the major rotamer is shown) δ 12.68 (s, 1H, N−H), 9.93 (s, 1H, N−H), 8.37 (s, 1H, H-8), 7.4−7.7 (broad, 4H, Ar−H_ortho, NH₂), 6.95 (d, 2H, *J* = 8.9 Hz, Ar−H_meta), 6.4 (broad, 1H, OH), 5.88 (d, 1H, *J* = 7.2

Hz, H-1′), 5.5 (broad, 1H, OH), 5.3 (broad, 1H, OH), 4.79 (m, 1H, H-2′), 4.18 (m, 1H, H-3′ or H-4′), 4.09 (m, 1H, H-4′ or H-3′), 3.76 (s, 3H, OCH$_3$), 3.4−3.7 (m, 2H, H-5′); [found M$^+$ + 1, 460.1673; C$_{18}$H$_{22}$N$_9$O$_6$ requires M, 460.1693]. Anal. Calcd (C$_{18}$H$_{21}$N$_9$O$_6$·2.2H$_2$O) C, H, N.

**2-[3-(2-Methoxyphenyl)aminocarbonyltriazene-1-yl]-adenosine 8i:** yield, 15.1 mg (60%), mp 162−164 °C; $^1$H NMR (DMSO-$d_6$) δ 12.65 (s, 1H, N−H), 9.03 (s, 1H, N−H), 8.41 (s, 1H, H-8), 8.04 (d, 1H, *J* = 7.8 Hz, Ar), 6.9−7.1 (broad, 4H, 3H−Ar, OH), 5.91 (d, 1H, *J* = 6.6 Hz, H-1′), 5.49 (m, 1H, OH), 5.21 (d, 1H, *J* = 4.3 Hz, OH), 4.67 (m, 1H, H-2′), 4.14 (m, 1H, H-3′ or H-4′), 3.99 (m, 1H, H-4′ or H-3′), 3.89 (s, 3H, OCH$_3$), 3.4−3.7 (m, 2H, H-5′); [found M$^+$ + 1, 460.1700; C$_{18}$H$_{22}$N$_9$O$_6$ requires M, 460.1693]. Anal. Calcd (C$_{18}$H$_{21}$N$_9$O$_6$·1.7H$_2$O) C, H, N.

**2-[3-(3-Trifluoromethylphenyl)aminocarbonyltriazene-1-yl]adenosine 8j:** yield, 19.0 mg (68%), mp 169−170 °C; $^1$H NMR (DMSO-$d_6$) (two rotamers are present in a ratio of 4:1; only the major rotamer is shown) δ 13.30 (s, 1H, N−H), 10.32 (s, 1H, N−H), 8.39 (s, 1H, H-8), 7.99 (m, 1H, Ar), 7.88 (broad, 2H, NH$_2$), 7.4−7.8 (broad, 3H, Ar), 6.52 (broad, 1H, OH), 5.89 (d, 1H, *J* = 7.2 Hz, H-1′), 5.48 (d, 1H, *J* = 6.3 Hz, OH), 5.28 (broad, 1H, OH), 4.79 (m, 1H, H-2′), 4.18 (m, 1H, H-3′ or H-4′), 4.11 (m, 1H, H-4′ or H-3′), 3.89 (s, 3H, OCH$_3$), 3.4−3.7 (m, 2H, H-5′); [found M$^+$ + 1, 498.1454; C$_{18}$H$_{19}$N$_9$O$_5$F$_3$ requires M, 498.1461]. Anal. Calcd (C$_{18}$H$_{18}$N$_9$O$_5$F$_3$·1.5H$_2$O) C, H, N.

**2-(3-Benzylaminocarbonyltriazene-1-yl)adenosine 8k:** yield, 30.0 mg (95%), mp 170−171 °C; $^1$H NMR (DMSO-$d_6$) δ 12.51 (s, 1H, N−H), 8.57 (m, 1H, N−H), 8.34 (s, 1H, H-8), 7.67 (s, 2H, NH$_2$), 7.37 (m, 5H, C$_6$H$_5$), 6.09 (d, 1H, *J* = 8.9 Hz, OH), 5.83 (d, 1H, *J* = 7.3 Hz, H-1′), 5.43 (d, 1H, *J* = 6.5 Hz, OH), 5.20 (d, 1H, *J* = 3.6 Hz, OH), 4.68 (m, 1H, H-2′), 4.43 (m, 2H, CH$_2$Ph), 4.03 (m, 1H, H-3′ or H-4′), 3.97 (m, 1H, H-4′ or H-3′), 3.3−3.5 (m, 2H, H-5′); [found M$^+$ + 1, 444.1705; C$_{18}$H$_{22}$N$_9$O$_5$ requires M, 444.1744]. Anal. Calcd (C$_{18}$H$_{21}$N$_9$O$_5$·1.6H$_2$O) C, H, N.

**2-[3-(2-Thiazolyl)aminocarbonyltriazene-1-yl]adenosine 8l:** yield, 17.1 mg (81%), mp 184−185 °C; $^1$H NMR (DMSO-$d_6$) δ 13.15 (broad, 1H, N−H), 12.50 (broad, 1H, N−H), 8.38 (s, 1H, H-8), 7.80 (s, 2H, NH$_2$), 7.52 (d, 1H, *J* = 3.4 Hz, thiazole), 7.26 (d, 1H, *J* = 3.4 Hz, thiazole), 7.0 (broad, 1H, OH), 5.88 (d, 1H, *J* = 7.5 Hz, H-1′), 5.46 (d, 1H, *J* = 6.4 Hz, OH), 5.28 (d, 1H, *J* = 3.4 Hz, OH), 4.78 (m, 1H, H-2′), 4.18 (m, 1H, H-3′ or H-4′), 4.14 (m, 1H, H-4′ or H-3′), 3.75−4.0 (m, 2H, H-5′); [found M$^+$ + 1, 437.1120; C$_{14}$H$_{17}$N$_{10}$O$_5$S requires M, 437.1104]. Anal. Calcd (C$_{14}$H$_{16}$N$_{10}$O$_5$S·2.9H$_2$O) C, H, N, S.

**2-(3-Benzoyltriazene-1-yl)adenosine 11.** A mixture of 2-nitrosoadenosine (29.6 mg, 0.10 mmol) and benzoylhydrazide (20.4 mg, 0.15 mmol) was stirred in a mixture of acetonitrile (2 mL) and acetic acid (0.2 mL) during 4 h at room temperature. The suspension was heated to dissolve solid material, cooled in ice, and filtered. Recrystallization from ethanol produced pure **11** (22.2 mg, 54%) as a yellow solid: mp 158−162°C; $^1$H NMR (DMSO-$d_6$) δ 13.5 (s, 1H, N−H), 8.45 (s, 1H, H-8), 8.06 (d, 2H, *J* = 7.5 Hz, Ar−H$_{ortho}$), 7.69 (s, 2H, NH$_2$), 7.5−7.7 (m, 3H, Ar), 5.92 (d, 1H, *J* = 6.0 Hz, H-1′), 5.50 (d, 1H, *J* = 6.1 Hz, OH), 5.20 (d, 1H, *J* = 4.8 Hz, OH), 5.14 (m, 1H, OH), 4.65 (m, 1H, H-2′), 4.17 (m, 1H, H-3′ or H-4′), 3.97 (m, 1H, H-4′ or H-3′), 3.5−3.7 (m, 2H, H-5′); [found M$^+$ + 1, 415.1478; C$_{17}$H$_{19}$N$_8$O$_5$ requires M, 415.1478]. Anal. Calcd (C$_{17}$H$_{18}$N$_8$O$_5$·2.4H$_2$O) C, H, N.

**2-(3-Phenyloxycarbonyltriazene-1-yl)adenosine 12.** A solution of 2-nitrosoadenosine (21.0 mg, 0.071 mmol) and phenyl carbazate (12.0 mg, 0.080 mmol) in a mixture of acetonitrile (2 mL), methanol (0.5 mL), and acetic acid (0.2 mL) was stirred during 24 h at room temperature. The suspension was diluted with dichloromethane and purified by flash chromatography with dichloromethane/methanol 80:20 as eluent. Evaporation of the solvents and trituration of the residue with ether gave **12** (11.2 mg, 32%) as a yellow solid: mp >180 °C (dec); $^1$H NMR (DMSO-$d_6$) δ 13.6 (broad, 1H, N−H), 8.44 (s, 1H, H-8), 7.67 (s, 2H, NH$_2$), 7.2−7.4 (m, 5H, Ar), 5.92 (d, 1H, *J* = 6.0 Hz, H-1′), 5.50 (d, 1H, *J* = 6.2 Hz, OH), 5.19 (d, 1H, *J* = 4.8 Hz, OH), 5.14 (m, 1H, OH), 4.63 (m,

1H, H-2′), 4.17 (m, 1H, H-3′ or H-4′), 3.97 (m, 1H, H-4′ or H-3′), 3.5−3.7 (m, 2H, H-5′); [found M$^+$ + 1, 431.1421; C$_{17}$H$_{19}$N$_8$O$_6$ requires M, 431.1428]. Anal. Calcd (C$_{17}$H$_{18}$N$_8$O$_6$·0.3H$_2$O) C, H, N.

**$N^6$-Phenyl-2′,3′,5′-tri-$O$-acetyl-2-nitroadenosine 13a.** Aniline (0.548 mL, 6.0 mmol) was added to a solution of **1** (0.915 g, 2.0 mmol) in DMF (4 mL). After 1 h of stirring, water (∼10 mL) was slowly added to precipitate the product. Filtration, washing with methanol, and drying in vacuo gave pure **13a** (1.01 g, 98%), as a yellow solid: mp 95−99 °C; $^1$H NMR δ 8.20 (s, 1H, H-8), 8.01 (s, 1H, N−H), 7.87 (d, 2H, *J* = 8.0 Hz, Ar−H$_{ortho}$), 7.45 (t, 2H, *J* = 8.0 Hz, Ar−H$_{meta}$), 7.21 (t, 1H, *J* = 8.0 Hz, Ar−H$_{para}$), 6.27 (d, 1H, *J* = 5.5 Hz, H-1′), 5.77 (m, 1H, H-2′), 5.63 (m, 1H, H-3′), 4.45−4.50 (m, 3H, H-4′, H-5′), 2.19, 2.14, and 2.10 (all 3H, s, acetates).

**$N^6$-Cyclopentyl-2′,3′,5′-tri-$O$-acetyl-2-nitroadenosine 13b.** Cyclopentylamine (99 μL, 1.02 mmol) and *N,N*-diisopropyl-ethylamine (0.262 mL, 1.5 mmol) were added to a solution of **1** (0.457 g, 1.0 mmol) in DMF (2 mL) at −18 °C. The bath was removed, and after 1 h of stirring at room temperature, the reaction mixture was purified by extractive workup (ether/water) to give crude **13b**, which was used without purification in the next step: $^1$H NMR δ 8.60 (broad, 1H, N−H), 8.05 (s, 1H, H-8), 6.20 (m, 1H, H-2′), 6.15 (d, 1H, *J* = 5.5 Hz, H-1′), 5.62 (m, 1H, H-3′), 4.7 (broad, *CH*-NH), 4.3−4.6 (m, 3H, H4′, H5′), 2.15, 2.10, and 2.07 (all 3H, s, acetates), 1.3−1.9 (m, 4H, cyclopentyl).

**$N^6$-(2,2-Diphenylethyl)-2′,3′,5′-tri-$O$-acetyl-2-nitroade-nosine 13c.** 2,2-Diphenylethylamine (0.197 g, 1.0 mmol) was reacted with **1** (1 mmol) as is described for **13b** to give crude **13c**, which was used without purification in the next step: $^1$H NMR δ 8.02 (s, 1H, H-8), 7.2−7.4 (m, 10H, Ar), 6.23 (m, 1H, H-2′), 6.19 (d, 1H, *J* = 5.5 Hz, H-1′), 5.72 (m, 1H, N−H), 5.62 (m, 1H, H-3′), 4.3−4.6 (m, 6H), 2.17, 2.11, and 2.08 (all 3H, s, acetates).

**$N^6$-Phenyl-2′,3′,5′-tri-$O$-acetyl-2-nitrosoadenosine 14a.** Nitropurine **13a** (0.514 g, 1.0 mmol) was hydrogenated with Pd/C (30 mg, 10%) in ethyl acetate (20 mL) during one night under 1 atm of H$_2$. The catalyst was removed by hot filtration over hyflow, and the filtrate was immediately oxidized with a solution of sodium periodate (0.321 g, 1.5 mmol) in water (10 mL) at 0 °C. The biphasic mixture was stirred vigorously during 1 h at this temperature, and after separation of the organic layer, the nitroso derivative **14a** (0.51 g, purity ∼ 90%) was obtained as a yellow glass: $^1$H NMR (concentration-dependent mixture of monomer and dimer; a sample of 1 mg in 0.5 mL of CDCl$_3$ shows predominantly the monomer) δ 8.31 (s, 1H, H-8), 7.93 (s, 1H, N−H), 7.86 (d, 2H, *J* = 7.6 Hz, Ar), 7.45 (t, 2H, *J* = 7.6 Hz, Ar), 7.19 (m, 1H, Ar), 6.44 (d, 1H, *J* = 5.5 Hz, H-1′), 5.90 (m, 1H, H-2′), 5.69 (m, 1H, H-3′), 4.4−4.5 (m, 3H, H-4′, H-5′), 2.20, 2.15, and 2.10 (all 3H, s, acetates).

**$N^6$-Cyclopentyl-2′,3′,5′-tri-$O$-acetyl-2-nitrosoadeno-sine 14b.** Nitro compound **13b** (0.10 g, 0.23 mmol) was reduced and oxidized as described for **14a** to give nitroso derivative **14b**, which was used without purification in the next step: $^1$H NMR δ 8.00 (broad, 2H, H-8, N−H), 6.33 (m, 1H, H-1′), 5.7 (m, 1H, H-2′), 5.5 (m, 1H, H-3′), 4.9 (broad, *CH*−NH), 4.3−4.6 (m, 3H, H4′, H5′), 2.18, 2.11, and 2.10 (all 3H, s, acetates), 1.3−1.9 (m, 4H, cyclopentyl).

**$N^6$-(2,2-Diphenylethyl)-2′,3′,5′-tri-$O$-acetyl-2-nitrosoad-enosine 14c.** Nitro compound **13c** (crude, from 1 mmol of **1**) was reduced and oxidized as described for **14a** to give nitroso derivative **14c**, which was used without purification in the next step: $^1$H NMR δ 8.12 (s, 1H, H-8), 7.0−7.3 (m, 10H, Ar), 6.23 (d, 1H, *J* = 5.5 Hz, H-1′), 5.90 (m, 1H, H-2′), 5.65 (m, 1H, H-3′), 4.3−4.6 (m, 6H), 2.20, 2.15, and 211 (all 3H, s, acetates).

**$N^6$-Phenyl-2-(3-aminocarbonyltriazene-1-yl)adeno-sine 16a.** The general procedure described for the synthesis of **7a−l** was used. Starting from **14a** (0.1 mmol) and **6a** (0.2 mmol), 32.2 mg of triacetate **15a** (58%) was isolated after chromatography with 5% methanol in ethyl acetate. The acetates were removed by stirring a solution of **15a** in methanol (2 mL) with aqueous ammonia (2 mL of a 25% solution) for 18 h at room temperature. The solvents were

removed in vacuo, and the residue was triturated with methanol (~2 mL), providing pure **16a** (19.0 mg, 76%): mp 187−88 °C; ^{1}H NMR (DMSO-$d_6$) $\delta$ 12.50 (s, 1H, N−H), 10.21 (s, 1H, N−H), 8.55 (s, 1H, H-8), 8.00 (d, 2H, $J$ = 8.1 Hz, Ar), 7.35 (m, 2H, Ar), 7.2 (broad, 2H, NH$_2$), 7.09 (m, 1H, Ar), 5.94 (d, 1H, $J$ = 7.0 Hz, H-1′), 5.81 (m, 1H, OH), 5.50 (d, 1H, $J$ = 6.3 Hz, OH), 5.26 (d, 1H, $J$ = 3.9, OH), 4.75 (m, 1H, H-2′), 4.17 (m, 1H, H-3′ or H-4′), 4.07 (m, 1H, H-4′ or H-3′), 3.5−3.8 (m, 2H, H-5′); [found M$^+$ + 1, 430.1591; C$_{17}$H$_{20}$N$_9$O$_5$ requires M, 430.1587]. Anal. Calcd (C$_{17}$H$_{19}$N$_9$O$_5$·1.1H$_2$O) C, H, N.

***N*$^6$-Phenyl-2-(3-phenylaminocarbonyltriazene-1-yl]-adenosine 16b.** The general procedure described for the synthesis of **7a−l** was used. Starting from **14a** (1.0 mmol) and 4-phenylsemicarbazide **6d** (2.0 mmol), a yield was obtained of 0.153 g of **15b** (24%) after chromatography with ethyl acetate. The acetates were removed by stirring a solution of **15b** in methanol (5 mL) with aqueous ammonia (5 mL of a 25% solution) for 18 h at room temperature. The solvents were removed in vacuo, and the residue was triturated with methanol (~2 mL), providing **16b** (0.062 g, 51%) as a yellow solid: mp 190−191 °C; ^{1}H NMR (DMSO-$d_6$) $\delta$ 12.97 (s, 1H, N−H), 10.28 (s, 1H, N−H), 10.05 (s, 1H, N−H), 8.56 (s, 1H, H-8), 8.00 (broad, 2H, Ar), 7.41 (broad, 2H, Ar), 7.37 (m, 4H, Ar), 7.10 (m, 2H, Ar), 6.34 (m, 1H, OH), 5.96 (d, 1H, $J$ = 7.2 Hz, H-1′), 5.87 (d, 1H, $J$ = 6.0 Hz, OH), 5.52 (m, 1H, OH), 4.84 (m, 1H, H-2′), 4.22 (m, 1H, H-3′ or H-4′), 4.13 (m, 1H, H-4′ or H-3′), 3.6−3.9 (m, 2H, H-5′); [found M$^+$ + 1, 506.1907; C$_{23}$H$_{24}$N$_9$O$_5$ requires M, 506.1901]. Anal. Calcd (C$_{23}$H$_{23}$N$_9$O$_5$·1.0H$_2$O) C, H, N.

***N*$^6$-Cyclopentyl-2-(3-phenylaminocarbonyltriazene-1-yl)adenosine 16c (TCPA).** The general procedure described for the synthesis of **7a−l** was used. Starting from **14b** (98 mg, 0.23 mmol) and 4-phenylsemicarbazide **6d** (0.4 mmol), a yield was obtained of 0.024 g of **15c** (0.039 mmol, 17% in four steps starting from **1**) after chromatography with ethyl acetate. The acetates were removed by stirring a solution of **15c** in methanol (2 mL) with aqueous ammonia (2 mL of a 25% solution) for 18 h at room temperature. The solvents were removed in vacuo, and the residue was triturated with methanol (~2 mL), providing **16c** (9.5 mg, 48%) as a yellow solid: mp 164−166 °C; ^{1}H NMR (DMSO-$d_6$) $\delta$ 12.81 (s, 1H, N−H), 10.17 (s, 1H, N−H), 8.37 (s, 1H, H-8), 8.21 (s, 1H, N−H), 7.59 (broad, 2H, Ar), 7.39 (t, 2H, $J$ = 7.7 Hz, Ar), 7.11 (m, 1H, Ar), 6.65 (m, 1H, OH), 5.88 (d, 1H, $J$ = 7.4 Hz, H-1′), 5.47 (d, 1H, $J$ = 5.8 Hz, OH), 5.26 (m, 1H, OH), 5.18 and 4.68 (m, 1H, C*H*NH, rotamers), 4.82 (m, 1H, H-2′), 4.19 (m, 1H, H-3′ or H-4′), 4.10 (m, 1H, H-4′ or H-3′), 3.65−3.9 (m, 2H, H-5′), 1.98 (m, 2H, cyclopentyl), 1.70 (m, 2H, cyclopentyl), 1.58 (m, 4H, cyclopentyl); [found M$^+$ + 1, 498.2207; C$_{22}$H$_{28}$N$_9$O$_5$ requires M, 498.2213]. Anal. Calcd (C$_{22}$H$_{27}$N$_9$O$_5$·0.5H$_2$O) C, H, N.

***N*$^6$-(2,2-Diphenylethyl)-2-(3-phenylaminocarbonyltriazene-1-yl)adenosine 16d.** The general procedure described for the synthesis of **7a−l** was used. Starting from **14c** (obtained from 1.0 mmol **1**) and 4-phenylsemicarbazide **6d** (1.4 mmol), a yield was obtained of 98 mg of **15d** (13% in four steps based on **1**) after chromatography with ethyl acetate. The acetates were removed by stirring a solution of **15d** in methanol (4 mL) with aqueous ammonia (4 mL of a 25% solution) for 18 h at room temperature. The solvents were removed in vacuo, and the residue was triturated with methanol (~2 mL), providing **16d** (54.3 mg, 69%) as a yellow solid: mp 138−140 °C; ^{1}H NMR (DMSO-$d_6$) $\delta$ 12.92 (s, 1H, N−H), 10.13 (s, 1H, N−H), 8.31 (s, 1H, H-8), 8.25 (s, 1H, N−H), 7.61 (broad, 2H, Ar), 7.0−7.4 (m, 13H, Ar), 6.49 (broad, 1H, OH), 5.86 (d, 1H, $J$ = 7.3 Hz, H-1′), 5.46 (d, 1H, $J$ = 6.1 Hz, OH), 5.26 (m, 1H, OH), 4.77 (m, 1H, H-2′), 4.67 and 4.61 (m, 1H, C*H*NH, rotamers), 4.18 (m, 3H, C*H*$_2$CH, H-3′ or H-4′), 4.08 (m, 1H, H-4′ or H-3′), 3.65−3.8 (m, 2H, H-5′); [found M$^+$ + 1, 610.2512; C$_{31}$H$_{32}$N$_9$O$_5$ requires M, 610.2527]. Anal. Calcd (C$_{31}$H$_{31}$N$_9$O$_5$·1.1H$_2$O) C, H, N.

**Improved Synthesis of TCPA (16c) via 17.** A mixture of *p*-nitrophenyl carbazate[18] (0.455 g, 2.31 mmol) and **14b** (prepared from 3.0 mmol **13b**) was stirred in a mixture of DCM (25 mL) and acetic acid (2.5 mL) during 18 h. Aqueous workup (saturated NaHCO$_3$, diethyl ether), drying (Na$_2$SO$_4$), and

evaporation of the solvents (bath temperature < 30 °C) gave crude **17**. Chromatography (silica, EtOAc) gave moderately pure **17** as a yellow glass (0.930 g, 46% based on **1**); ^{1}H NMR (DMSO-$d_6$) $\delta$ 12.0 (s, 1H, N−H), 8.32 (d, 2H, $J$ = 9.0 Hz, ArH), 7.96 (s, 1H, H-8), 7.49 (d, 2H, $J$ = 9.0 Hz, ArH), 6.93 (d, 1H, $J$ = 7.3 Hz, N−H), 6.20 (d, 1H, $J$ = 3.9 Hz, H1′), 5.83 (m, 1H, H-2′), 5.74 (m, 1H, H-3′), 4.65 (broad, 1H, C*H*NH), 4.45 (m, 3H, H4′, H5′), 2.09, 2.07 and 2.04 (all 3H, s, acetates), 1.5−1.8 (m, 8H, cyclopentyl).

A solution of **17** (0.335 g, 0.50 mmol) was stirred with aniline (0.456 mL, 5.0 mmol) in anhydrous acetonitrile (10 mL) during 8 h. Evaporation of the solvent and chromatography (silica, ethyl acetate) gave **15c** (0.259 g, 0.416 mmol, 83%) as a yellow glass: ^{1}H NMR $\delta$ 14.5 (broad, 1H, N−H), 9.7 (broad, 1H, N−H), 8.51 (s, 1H, H-8), 7.93 (broad, 1H, N−H), 7.60 (broad, 2H, Ar), 7.36 (t, 2H, $J$ = 7.7 Hz, Ar), 7.13 (m, 1H, Ar), 6.16 (m, 1H, H-1′), 5.98 (m, 1H, H-2′), 5.75 (m, 1H, H3′), 4.49 (broad, 1H, C*H*NH), 4.3−4.5 (m, 3H, H4′, H5′), 2.12, 2.06, 2.04 (all 3H, s, acetates), 1.5−1.8 (m, 8H, cyclopentyl). The acetates were removed by stirring a solution of **15c** (0.238 g, 0.38 mmol) in methanol (10 mL) with aqueous ammonia (10 mL of a 25% solution) for 18 h at room temperature. The solvents were removed in vacuo, and the residue was triturated with methanol (~5 mL), providing **16c** (0.116 g, 0.23 mmol, 61%) as a yellow solid.

**Stability Studies.** A solution of **8d** or **16c** (0.5 mg) in DMSO-$d_6$ (50 $\mu$L) was diluted with phosphate buffer in D$_2$O (450 $\mu$L, pD 7.8, 0.05 M) and kept in an NMR tube at 25 °C. ^{1}H NMR spectra were obtained at 2 h intervals for 5 days. No changes in the spectrum were observed.

**Hydrolysis of 8d.** A solution of **8d** in a mixture of DMSO-$d_6$ (450 $\mu$L) and D$_2$O (50 $\mu$L) was heated in an NMR tube at 80 °C. After 2 h, complete conversion was observed to aniline and 2-aminoadenosine (**18**): ^{1}H NMR $\delta$ 7.93 (s, 1H, H-8), 7.01 (m, 2H, aniline), 6.58 (t, 2H, $J$ = 7.3 Hz, aniline), 6.53 (t, $J$ = 7.3 Hz, aniline), 5.70 (d, 1H, $J$ = 6.4 Hz, H-1′), 4.51 (m, 1H, H-2′), 3.64 (dd, 1H, $J$ = 12.2 Hz, $J$ = 3.2 Hz, H-5a′), 3.54 (dd, 1H, $J$ = 12.2 Hz, $J$ = 3.2 Hz, H-5b′).

**Hydrolysis of 16c.** Hydrolysis of **16c** was performed as described for **8d** to give 2-amino-CPA **19** and aniline: ^{1}H NMR $\delta$ 7.89 (s, 1H, H-8), 7.01 (m, 2H, aniline), 6.58 (t, 2H, $J$ = 7.3 Hz, aniline), 6.53 (t, $J$ = 7.3 Hz, aniline), 5.70 (d, 1H, $J$ = 6.4 Hz, H-1′), 4.50 (m, 1H, H-2′), 3.9−4.1 (m, 3H, H-3′, H-4′, C*H*NH), 3.64 (dd, 1H, $J$ = 12.3 Hz, $J$ = 3.2 Hz).

**Cell Cultures.** Radioligand binding studies were performed on stably transfected cell lines expressing human adenosine receptors. CHO cells expressing the human adenosine A$_1$ receptor were obtained from Dr. A. Townsend-Nicholson. These cells were cultured at 37 °C in a 5% CO$_2$ atmosphere in a 1:1 mixture of DMEM/F12, 2 mM Glutamax (a stable analogue of glutamine), 10% newborn calf serum with 50 IU/mL penicillin, and 50 $\mu$g/mL streptomycin. Dr. S. Rees kindly provided CHO cells expressing the human A$_{2A}$ or human A$_{2B}$ receptor. These cells were cultured at 37 °C in a 5% CO$_2$ atmosphere in a 1:1 mixture of DMEM/F12, 2 mM Glutamax (a stable analogue of glutamine), 10% newborn calf serum, 1 mg/mL G418 with 50 IU/mL penicillin, and 50 $\mu$g/mL streptomycin. HEK293 cells expressing human adenosine A$_3$ receptors were from Dr. K.-N. Klotz. These cells were cultured at 37 °C in a 7% CO$_2$ atmosphere in DMEM, 2 mM Glutamax (a stable analogue of glutamine), 10% newborn calf serum, 0.5 mg/mL G418, with 50 IU/mL penicillin, and 50 $\mu$g/mL streptomycin.

Confluent cells expressing the human A$_1$, A$_{2A}$, or A$_{2B}$ receptor or semiconfluent cells expressing the human A$_3$ adenosine receptor were trypsinized and centrifuged for 10 min at 1000 rpm. The cell pellet of CHO cells expressing the adenosine A$_{2B}$ receptor was resuspended in 140 mM NaCl, 5 mM KCl, and 5 mM glucose in 20 mM Tris-HCl adjusted to pH 7.4 at room temperature. The other cell pellets were resuspended in 50 mM Tris-HCl, pH 7.4, at room temperature and homogenized on ice for 5 s at position 8 with an Ystral. The homogenate was centrifuged for 45 min at 12700 rpm in an SW-30 rotor at 4 °C. The resulting pellet was resuspended

in 50 mM Tris-HCl, pH 7.4, at room temperature. Adenosine deaminase, 2 IU/mL, was added, and aliquots were stored at $-80$ °C.

**Radioligand Binding Studies.** Stock solutions of ligands were made in DMSO. The final concentration of DMSO in the assay did not exceed 1%. [³H]DPCPX and [¹²⁵I]IAB-MECA were obtained from Amersham, and [³H]ZM241385 was from Tocris Cookson Ltd. (Northpoint, U.K.). $IC_{50}$ values were estimated by GraphPad Prism software (GraphPad, San Diego, CA). $IC_{50}$ values obtained from the competition curves were converted to $K_i$ values using the Cheng−Prusoff equation.

**Adenosine $A_1$ Receptor.** Membranes, containing 40 μg of protein, were incubated in a total volume of 400 μL of 50 mM Tris-HCl, pH 7.4, at room temperature with [³H]DPCPX (final concentration = 1.6 nM) during 1 h at 25 °C in a shaking water bath. Nonspecific binding was determined in the presence of 10 μM CPA. The incubation was terminated by filtration over Whatman GF/B filters under reduced pressure with a Brandell harvester. Filters were washed three times with ice-cold buffer and placed in scintillation vials. Emulsifier Safe, 3.5 mL, was added, and after 2 h, radioactivity was counted in an LKB rackbeta scintillation counter.

**Adenosine $A_{2A}$ Receptor.** Membranes, containing 40 μg of protein, were incubated in a total volume of 400 μL of 50 mM Tris-HCl, pH 7.4, at room temperature with [³H]-ZM241385 (final concentration = 2.0 nM) during 2 h at 25 °C in a shaking water bath. Nonspecific binding was determined in the presence of 100 μM CPA. The incubation was terminated by filtration over Whatman GF/B filters under reduced pressure with a Brandell harvester. Filters were washed four times with ice-cold buffer and placed in scintillation vials. Emulsifier Safe, 3.5 mL, was added, and after 2 h, radioactivity was counted in an LKB rackbeta scintillation counter.

**Adenosine $A_{2B}$ Receptor.** Cells (500000 per assay) were incubated in a total volume of 100 μL of 140 mM NaCl, 5 mM KCl, 5 mM glucose, and 20 mM Tris-HCl, pH 7.4, at room temperature with [³H]DPCPX (final concentration = 6.0 nM) during 3 h at 25 °C in a shaking water bath. Nonspecific binding was determined in the presence of 10 μM CGS15943. The incubation was terminated by filtration over Whatman GF/B filters under reduced pressure with a Millipore manifold. Filters were washed eight times with 2 mL of ice-cold buffer and placed in scintillation vials. Emulsifier Safe, 3.5 mL, was added, and after 2 h, radioactivity was counted in an LKB rackbeta scintillation counter.

**Adenosine $A_3$ Receptor.** Membranes, containing 20−40 μg of protein, were incubated in a total volume of 100 μL of 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM EDTA, and 0.01% CHAPS, pH 7.4, at room temperature with [¹²⁵I]IAB-MECA (final concentration = 0.10 nM) during 1 h at 37 °C in a shaking water bath. Nonspecific binding was determined in the presence of 100 μM R-PIA. The incubation was terminated by filtration over Whatman GF/B filters under reduced pressure with a Brandell harvester. Filters were washed three times with ice-cold buffer and placed in vials. Radioactivity was counted in a gamma counter.

**cAMP Experiments.** The CHO cells stably expressing the human adenosine $A_1$ receptor from Dr. A. Townsend-Nicholson were seeded in 24 well plates at a density of $2 \times 10^5$ cells/well. The next day cAMP generation was performed as previously described.[26] Inhibition of 10 μM forskolin-induced cAMP formation by CCPA and TCPA was determined by competition with [³H]cAMP for binding to protein kinase A.[26] The data reflect three independent experiments, performed in duplicate. To analyze the data, PRISM software (GraphPad, San Diego, CA) was used.

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